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FLEA OCTOPAMINE RECEPTOR NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

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SPECIFICATION

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FLEA OCTOPAMINE RECEPTOR NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

Field of the Invention

[0001] The present invention relates to flea octopamine receptor nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules, antibodies, and inhibitory compounds. The present invention also includes therapeutic compositions comprising such inhibitors, as well as uses thereof.

Background of Invention

[0002] Flea infestation of animals is a health and economic concern for pet owners. In particular, the bites of fleas are a problem for animals maintained as pets because the infestation becomes a source of annoyance not only for the pet but also for the pet owner who may find his or her home generally contaminated with insects. Fleas are known to directly cause a variety of diseases, including allergy, and also carry a variety of infectious agents including, but not limited to, endoparasites (e.g., nematodes, cestodes, trematodes and protozoa), bacteria and viruses. As such, fleas are a problem not only when they are on an animal but also when they are in the general environment of the animal.

[0003] The medical importance of flea infestation has prompted the development of reagents capable of controlling flea infestation. Commonly encountered methods to control flea infestation are generally focused on use of insecticides, which are often unsuccessful for one or more of the following reasons: (1) failure of owner compliance

(frequent administration is required); (2) behavioral or physiological intolerance of the pet to the pesticide product or means of administration; and (3) the emergence of flea populations resistant to the prescribed dose of pesticide.

[0004] Octopamine receptor is a member of the biogenic amine receptor family, which also includes receptors for dopamine, serotonin, and tyramine. Octopamine is a major neuromodulator in insects with neurotransmitter and neurohormone functions and is a known activator of adenylate cyclase, resulting in stimulation of cyclic AMP production.

[0005] Prior investigations have described certain insect biogenic amine receptors in *Drosophila melanogaster*, including for example, Arakawa et al. 1990, *Neuron*, 2:343-354, Venter et al., U.S. Patent Number 5,474,898, Saudou et al., 1990, *EMBO Journal*, 9(11):3611-3617, and Han et al., 1998, *J. Neuroscience*, 18(10):3650-3658. Unfortunately, cDNA's encoding members of the biogenic amine receptor family have proven difficult to clone due to the rarity of the sequence as cDNA and often have highly related sequences and activities, which has resulted in confusion in the art with respect to the true identity of reported sequences for any given biogenic amine receptor. For example, the biogenic amine receptor identified as an octopamine receptor by Arakawa et al. was later determined to actually represent a tyramine receptor.

[0006] Insect octopamine receptor is a known target of various insecticides, including formamadine compounds such as demethylchlordimeform (DCDM). However, no formamadine compound to date has been shown to be safe and efficacious for use for treating flea infestations on a host animal. Octopamine receptor is not present in vertebrates and within insects susceptibility to insecticides targeting the octopamine receptor have been shown to vary by species. Therefore, in order to create compounds and treatments which are efficacious against fleas while minimizing toxicity to the host animal or to non-target insects, it would be a distinct advantage to have the sequence of the flea octopamine receptor. Accordingly, isolation and sequencing of flea octopamine receptor genes may be critical for use in identifying specific agents for treating animals for flea infestation.

Summary of Invention

[0007] The present invention provides flea octopamine receptor proteins; nucleic acid molecules encoding flea octopamine receptor proteins; antibodies raised against such proteins (i.e., anti-flea octopamine receptor antibodies); mimetopes of such proteins or antibodies; and compounds that inhibit flea octopamine receptor activity (i.e. inhibitory compounds or inhibitors).

[0008] The present invention also includes methods to obtain such proteins, mimetopes, nucleic acid molecules, antibodies and inhibitory compounds. The present invention also includes the use of proteins and antibodies to identify such inhibitory compounds as well as assay kits to identify such inhibitory compounds. Also included in the present invention are therapeutic compositions comprising proteins, mimetopes, nucleic acid molecules, antibodies and inhibitory compounds of the present invention including therapeutic compounds derived from a protein of the present invention that inhibit the activity of flea octopamine receptor proteins.

[0009] One embodiment of the present invention is an isolated flea octopamine receptor nucleic acid molecule of at least 35 nucleotides that hybridizes with a nucleic acid sequence having SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:13, under conditions that allow less than or equal to 5% base pair mismatch, wherein such nucleic acid molecule encodes a protein that binds octopamine, or a nucleic acid molecule having a sequence fully complementary to such a nucleic acid molecule. Another embodiment of the present invention is an isolated flea octopamine receptor nucleic acid molecule having a nucleic acid sequence that is at least 95% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 or SEQ ID NO:13, or a fragment thereof having at least 35 nucleotides that encodes a protein that binds octopamine. Another embodiment of the present invention is an isolated flea octopamine receptor nucleic acid molecule that encodes a protein that is at least 95% identical to SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, or a fragment thereof having at least 35 nucleotides that encodes a protein that binds octopamine, or a nucleic acid sequence fully complementary to such a nucleic acid sequence.

[0010] The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include a nucleic acid molecule of the present invention.

Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells. Also included are methods to produce a protein of the present invention.

[0011] Another embodiment of the present invention includes an isolated flea octopamine receptor protein that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, wherein such protein binds to octopamine.

[0012] Another embodiment of the present invention includes an isolated flea octopamine receptor protein encoded by a nucleic acid molecule that hybridizes with a nucleic acid sequence having SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:13, under conditions that allow less than or equal to 5% base pair mismatch.

[0013] Another embodiment of the present invention includes a method to detect an inhibitor of flea octopamine receptor activity, said method comprising (a) contacting an isolated flea octopamine receptor protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has flea octopamine receptor protein activity, and (b) determining if said putative inhibitory compound inhibits flea octopamine receptor protein activity.

Detailed Description

[0014]

The present invention provides for flea octopamine receptor nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. As used herein, flea octopamine receptor nucleic acid molecules and proteins encoded by such nucleic acid molecules are also referred to as octopamine receptor nucleic acid molecules and proteins of the present invention, respectively. Flea octopamine receptor nucleic acid molecules and proteins of the present invention can be isolated from a flea or prepared recombinantly or synthetically. Flea octopamine receptor nucleic acid molecules of the present invention can be RNA or DNA, or modified forms thereof, and can be double-stranded or single-stranded, examples of nucleic acid molecules include, but are not limited to, complementary DNA (cDNA) molecules, genomic DNA molecules, synthetic DNA molecules, DNA molecules which are specific tags for messenger RNA, and

corresponding mRNA molecules. As such, a flea nucleic acid molecule of the present invention is not intended to refer to an entire chromosome within which such a nucleic acid molecule is contained, however, a flea octopamine receptor nucleic acid molecule of the present invention may include all regions such as regulatory regions that control production of flea octopamine receptor proteins encoded by such a nucleic acid molecule (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, the phrase flea octopamine receptor protein refers to a protein encoded by a flea octopamine receptor nucleic acid molecule.

[0015] Flea octopamine receptor nucleic acid molecules of known length isolated from a flea, such as *Ctenocephalides felis* are denoted nCfOCR $_{\#}$, for example nCfOCR $_{2136}$, wherein # refers to the number of nucleotides in that molecule, and flea octopamine receptor proteins of known length are denoted PCfOCR $_{\#}$ (for example PCfOCR $_{712}$) wherein # refers to the number of amino acid residues in that molecule.

[0016] The present invention also provides for flea octopamine receptor DNA molecules that are specific tags for messenger RNA molecules. Such DNA molecules can correspond to an entire or partial sequence of a messenger RNA, and therefore, a DNA molecule corresponding to such a messenger RNA molecule (i.e. a cDNA molecule), can encode a full-length or partial-length protein. A nucleic acid molecule encoding a partial-length protein can be used directly as a probe or indirectly to generate primers to identify and/or isolate a cDNA nucleic acid molecule encoding a corresponding, or structurally related, full-length protein. A cDNA encoding a partial-length octopamine receptor protein can also be used in a similar manner to identify a genomic nucleic acid molecule, such as a nucleic acid molecule that contains the complete gene including regulatory regions, exons and introns. Methods for using cDNA molecules and sequences encoding partial-length flea octopamine receptor proteins to isolate nucleic acid molecules encoding full-length flea octopamine receptor proteins and corresponding cDNA molecules are described in the examples herein below.

[0017] The proteins and nucleic acid molecules of the present invention can be obtained from their natural source, or can be produced using, for example, recombinant nucleic

acid technology or chemical synthesis. Also included in the present invention is the use of these proteins and nucleic acid molecules as well as antibodies and inhibitory compounds thereto as therapeutic compositions, as well as in other applications, such as those disclosed below.

[0018] One embodiment of the present invention is an isolated protein that includes a flea octopamine receptor protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein, a nucleic acid molecule, an antibody and a therapeutic composition refers to one or more or at least one protein, nucleic acid molecule, antibody and therapeutic composition respectively. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising, "including, and "having can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

[0019] As used herein, isolated flea octopamine receptor proteins of the present invention can be full-length proteins or any homologue of such proteins. An isolated protein of the present invention, including a homologue, can be identified in a straight-forward manner by the protein"s ability to elicit an immune response against a flea octopamine receptor protein or by the protein"s ability to exhibit flea octopamine receptor activity, e.g. the ability to bind to octopamine. Examples of flea octopamine receptor homologue proteins include flea octopamine receptor proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homologue includes at least one epitope capable of eliciting an immune response against a flea octopamine receptor protein, and/or of binding to an antibody directed against a flea octopamine receptor protein. That is, when the homologue is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will

produce an immune response against at least one epitope of a natural flea octopamine receptor protein. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term epitope refers to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody or a T cell receptor. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about four to six amino acids. As is appreciated by those skilled in the art, an epitope can include amino acids that naturally are contiguous to each other as well as amino acids that, due to the tertiary structure of the natural protein, are in sufficiently close proximity to form an epitope. According to the present invention, an epitope includes a portion of a protein comprising at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids or at least 50 amino acids in length.In one embodiment of the present invention a flea octopamine receptor homologue protein has flea octopamine receptor activity, i.e. the homologue exhibits an activity similar to its natural counterpart, e.g. the ability to bind octopamine. Methods to detect and measure such activities are known to those skilled in the art.

[0020] Flea octopamine receptor homologue proteins can be the result of natural allelic variation or natural mutation. Flea octopamine receptor protein homologues of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

[0021] Flea octopamine receptor proteins of the present invention are encoded by flea octopamine receptor nucleic acid molecules. As used herein, flea octopamine receptor nucleic acid molecules include nucleic acid sequences related to natural flea octopamine receptor genes, and, preferably, to *C. felis* flea octopamine receptor genes. As used herein, flea octopamine receptor genes include all regions such as regulatory regions that control production of flea octopamine receptor proteins encoded by such genes (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-

translated coding regions. As used herein, a nucleic acid molecule that includes or comprises a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons,such as is often found for a flea gene. As used herein, the term coding region refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that coding region that is translated into a full-length, i.e., a complete protein as would be initially translated in its natural millieu, prior to any post-translational modifications.

[0022] One embodiment of the present invention is a *C. felis* flea octopamine receptor gene that includes the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13 either in a consecutive array or interrupted by naturally occurring introns. These nucleic acid sequences are further described herein. For example, nucleic acid sequence SEQ ID NO:11 represents the deduced sequence of the coding strand of a *C. felis* cDNA denoted herein as *C. felis* octopamine receptor nucleic acid molecule nCfOCR$_{2136}$, the production of which is disclosed in the Examples. Nucleic acid molecule SEQ ID NO:11 comprises an apparently full-length coding region. The complement of SEQ ID NO:11 (represented herein by SEQ ID NO:13) refers to the nucleic acid sequence of the strand fully complementary to the strand having SEQ ID NO:11, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a complete double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:11 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding a flea octopamine receptor protein of the present invention.

[0023] Translation of SEQ ID NO:11, the coding strand of nCfOCR$_{2136}$ yields a protein of 712 amino acids, denoted herein as PCfOCR$_{712}$, the amino acid sequence of which is presented in SEQ ID NO:12, assuming an (a) initiation codon extending from nucleotide 1 to 3 of SEQ ID NO:11 and (b) a last codon extending from nucleotide 2134 to 2136 of SEQ ID NO:11.

[0024] In one embodiment, a gene or other nucleic acid molecule of the present invention can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13. For example, an allelic variant of a *C. felis* octopamine receptor gene including SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants (i.e. alleles corresponding to, or of, cited nucleic acid sequences) usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to occur naturally within a given flea species, since the genome is diploid, and sexual reproduction will result in the reassortment of alleles.

[0025] In one embodiment of the present invention, isolated flea octopamine receptor proteins are encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to genes or other nucleic acid molecules encoding flea octopamine receptor proteins, respectively. The minimal size of flea octopamine receptor proteins of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridizing under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the flea octopamine receptor nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a

given nucleic acid molecule or are clustered (i.e.,in distinct regions on a given nucleic acid molecule.

[0026] The minimal size of a nucleic acid molecule capable of forming a stable hybrid with a gene encoding a flea octopamine receptor protein is at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. The minimal size of a nucleic acid molecule used to encode a flea octopamine receptor protein homologue of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of flea octopamine receptor protein homologues of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule encoding a flea octopamine receptor protein of the present invention because a nucleic acid molecule of the present invention can include a portion of a gene or cDNA or RNA, an entire gene or cDNA or RNA, or multiple genes or cDNA or RNA. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

[0027] Stringent hybridization conditions are determined based on defined physical properties of the flea octopamine receptor nucleic acid molecule to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.* , 1989, *Molecular Cloning: A Laboratory Manual* , Cold Spring Harbor Labs Press, and Meinkoth, *et al.,* 1984, *Anal. Biochem. 138* , 267each of which is incorporated by reference herein in its entirety. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (° C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150

nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands: $T_m = 81.5\ ^\circ C + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61$ (%formamide).

[0028] For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation: $T_d = 4(G + C) + 2(A + T)$.

[0029] A temperature of 5 ° C below T_d is used to detect hybridization between perfectly matched molecules.

[0030] Also well known to those skilled in the art is how base pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1 ° C for each 1% of mismatched base pairs for hybrids greater than about 150 bp, and T_d decreases about 5 °C for each mismatched base pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with greater than a specified % base pair mismatch will hybridize. Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under conditions designed to allow a

desired amount of base pair mismatch.

[0031] Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

[0032] For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under conditions that would allow less than or equal to 30% pair mismatch with a flea octopamine receptor nucleic acid molecule of about 150 bp in length or greater, the following conditions could preferably be used. The average G + C content of flea DNA is about 37%, as calculated from known flea nucleic acid sequences. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of about 37 ^{o} C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. The skilled artisan would calculate the washing conditions required to allow up to 30% base pair mismatch. For example, in a wash solution comprising 1X SSC in the absence of nucleic acid helix destabilizing compounds, the T_m of perfect hybrids would be about 79.6 ^{o} C:81.5 ^{o} C + 16.6 log (.15M) + (0.41 x 37) - (500/150) - (0.61 x 0) = 79.6 ^{o} C.

[0033] Thus, to achieve hybridization with nucleic acid molecules having about 20% base pair mismatch, hybridization washes would be carried out at a temperature of less than or equal to 59.6 ^{o} C. It is thus within the skill of one in the art to calculate

additional hybridization temperatures based on the desired percentage base pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present invention having sequences specified herein becomes longer than 150 nucleotides, the T_m for a hybridization reaction allowing up to 20% base pair mismatch will not vary significantly from 59.6 E C. Similarly, to achieve hybridization with nucleic acid molecules having about 10% base pair mismatch, hybridization washes would be carried out at a temperature of less than or equal to 69.6 ^{o} C and to achieve hybridization with nucleic acid molecules having about 5% base pair mismatch, hybridization washes would be carried out at a temperature of less than or equal to 74.6 ^{o} C.

[0034]

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid or protein sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules or proteins. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, the SeqLab ® Wisconsin Package ™ Version 10.0-UNIX sequence analysis software, available from Genetics Computer Group, Madison, WI (hereinafter SeqLab); and DNAsis ® sequence analysis software, version 2.0, available from Hitachi Software, San Bruno, CA (hereinafter DNAsis). Such software programs represent a collection of algorithms paired with a graphical user interface for using the algorithms. The DNAsis and SeqLab software, for example, employ a particular algorithm, the Needleman-Wunsch algorithm to perform pair-wise comparisons between two sequences to yield a percentage identity score, see Needleman, S.B. and Wunch, C.D., 1970, *J. Mol. Biol.* , 48, 443, which is incorporated herein by reference in its entirety. Such algorithms, including the Needleman-Wunsch algorithm, are commonly used by those skilled in the nucleic acid and amino acid sequencing art to compare sequences. A preferred method to determine percent identity among amino

acid sequences and also among nucleic acid sequences includes using the Needleman-Wunsch algorithm, available in the SeqLab software, using the Pairwise Comparison/Gap function with the nwsgapdna.cmp scoring matrix, the gap creation penalty and the gap extension penalties set at default values, and the gap shift limits set at maximum (hereinafter referred to as SeqLab default parameters). An additional preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Higgins-Sharp algorithm, available in the DNAsis software (hereinafter DNAsis), with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 5, and the floating gap penalty set at 10. A particularly preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Needleman-Wunsch algorithm available in the SeqLab software, using the SeqLab default parameters.

[0035] One embodiment of the present invention includes a flea octopamine receptor protein. A preferred flea octopamine receptor protein includes a protein encoded by a nucleic acid molecule that hybridizes under conditions that preferably allow less than or equal to 30% base pair mismatch, preferably under conditions that allow less than or equal to 20% base pair mismatch, preferably under conditions that allow less than or equal to 10% base pair mismatch, preferably under conditions that allow less than or equal to 8% base pair mismatch, preferably under conditions that allow less than or equal to 5% base pair mismatch or preferably under conditions that allow less than or equal to 2% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:13.

[0036] Another embodiment of the present invention includes a flea octopamine receptor protein encoded by a nucleic acid molecule that hybridizes under conditions comprising, (a) hybridizing in a solution comprising 1X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of 37 ° C and (b) washing in a solution comprising 1X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of 49.6 ° C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:13.

[0037] Another preferred flea octopamine receptor protein of the present invention includes a protein that is encoded by a nucleic acid molecule that is preferably at least 70%, preferably at least 80%, preferably at least 90% identical, preferably at least 92% identical, preferably at least 95% identical or preferably at least 98% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11; also preferred are fragments (i.e. portions) of such proteins encoded by nucleic acid molecules that are at least 35 nucleotides. Percent identity as used herein is determined using the Needleman-Wunsch algorithm, available in the SeqLab software using default parameters.

[0038] Additional preferred flea octopamine receptor proteins of the present invention include proteins having the amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, and proteins comprising homologues of a protein having the amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, wherein such a homologue comprises at least one epitope that elicits an immune response against a protein having an amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12. Likewise, also preferred are proteins encoded by nucleic acid molecules comprising nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11, or by homologues thereof.

[0039] A preferred isolated flea octopamine receptor protein of the present invention is a protein encoded by at least one of the following nucleic acid molecules: $nCfOCR_{111}$, $nCfOCR_{2061}$, $nCfOCR_{868}$, and $nCfOCR_{2136}$, or allelic variants of any of these nucleic acid molecules. Also preferred is an isolated protein encoded by a nucleic acid molecule having nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11; or a protein encoded by an allelic variant of any of these listed nucleic acid molecules.

[0040]

Preferred flea octopamine receptor proteins of the present invention include proteins having amino acid sequences that are at least 70%, preferably 80%, preferably 90%, preferably 95%, preferably at least 98%, preferably at least 99%, or preferably 100% identical to amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12; and proteins encoded by allelic variants of nucleic acid molecules encoding flea octopamine receptor proteins having amino acid sequences SEQ ID NO:4, SEQ ID NO:7

and SEQ ID NO:12.

[0041] Preferred flea octopamine receptor proteins of the present invention include proteins selected from the group consisting of (a) a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12; and (b) a protein comprising an at least 40 contiguous amino acid portion identical in sequence to an at least 40 contiguous amino acid portion of an amino acid sequence of (a).

[0042] In one embodiment of the present invention, *C. felis* octopamine receptor proteins comprise amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12 (including, but not limited to, the proteins consisting of amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, fusion proteins and multivalent proteins), and proteins encoded by allelic variants of nucleic acid molecules encoding proteins having amino acid sequence SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12.

[0043] In one embodiment, a preferred flea octopamine receptor protein comprises an amino acid sequence of at least 35 amino acids, preferably at least 50 amino acids, preferably at least 100 amino acids, preferably at least 125 amino acids, preferably at least 150 amino acids, preferably at least 175 amino acids, preferably at least 180 amino acids, preferably at least 190 amino acids, preferably at least 200 amino acids, preferably at least 225 amino acids, preferably at least 250 amino acids, preferably at least 275 amino acids, preferably at least 300 amino acids, preferably at least 350 amino acids, preferably at least 400 amino acids, preferably at least 450 amino acids, preferably at least 500 amino acids, preferably at least 550 amino acids, preferably at least 600 amino acids, preferably at least 650 amino acids, or preferably at least 690 amino acids. In another embodiment, preferred flea octopamine receptor proteins comprise full-length proteins, i.e., proteins encoded by full-length coding regions, or post-translationally modified proteins thereof, such as mature proteins from which initiating methionine and/or signal sequences or pro sequences have been removed.

[0044] Also preferred are flea octopamine receptor proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11, as well as allelic variants of these nucleic acid molecules. A portion of such flea octopamine receptor nucleic acid molecule is

preferably at least 35 nucleotides in length.

[0045] In another embodiment, a preferred flea octopamine receptor protein of the present invention is encoded by a nucleic acid molecule comprising at least 30 nucleotides, preferably at least 50 nucleotides, preferably at least 75 nucleotides, preferably at least 100 nucleotides, preferably at least 125 nucleotides, preferably at least 150 nucleotides, preferably at least 175 nucleotides, preferably at least 200 nucleotides, preferably at least 250 nucleotides, preferably at least 350 nucleotides, preferably at least 450 nucleotides, preferably at least 550 nucleotides, preferably at least 650 nucleotides, preferably at least 750 nucleotides, preferably at least 1000 nucleotides, preferably at least 1500 nucleotides, preferably at least 1750 nucleotides, preferably at least 2000 nucleotides or preferably at least 2050 nucleotides in length that bind octopamine. Preferred flea octopamine receptor proteins of the present invention are encoded by nucleic acid molecules comprising apparently full-length flea octopamine receptor coding region, i.e., nucleic acid molecules encoding an apparently full-length flea octopamine receptor protein, or extracellular domain.

[0046] Preferred flea octopamine receptor proteins of the present invention can be used to develop inhibitors that, when administered to an animal in an effective manner, are capable of protecting that animal from flea infestation. In accordance with the present invention, the ability of an inhibitor of the present invention to protect an animal from flea infestation refers to the ability of that inhibitor to, for example, treat, ameliorate and/or prevent infestation caused by fleas. In particular, the phrase "to protect an animal from flea infestation" refers to reducing the potential for flea population expansion on and around the animal (i.e., reducing the flea burden). Preferably, the flea population size is decreased, optimally to an extent that the animal is no longer bothered by fleas. A host animal, as used herein, is an animal from which fleas can feed by attaching to and feeding through the skin of the animal. Fleas, and other ectoparasites, can live on a host animal for an extended period of time or can attach temporarily to an animal in order to feed. At any given time, a certain percentage of a flea population can be on a host animal whereas the remainder can be in the environment of the animal. Such an environment can include not only adult fleas, but also flea eggs and/or flea larvae. The environment can be of any size such that fleas in the environment are able to jump onto and off of a host animal. For example, the

environment of an animal can include plants, such as crops, from which fleas infest an animal. As such, it is desirable not only to reduce the flea burden on an animal per se, but also to reduce the flea burden in the environment of the animal.

[0047] Suitable fleas to target include any flea that is essentially incapable of causing disease in an animal administered an inhibitor of the present invention. As such, fleas to target include any flea that produces a protein that can be targeted by an inhibitory compound that inhibits a flea flea octopamine receptor protein function, thereby resulting in the decreased ability of the parasite to cause disease in an animal. Preferred fleas to target include fleas of the following genera: *Ctenocephalides* , *Cyopsyllus* , *Diamanus* (*Oropsylla)* , *Echidnophaga* , *Nosopsyllus* , *Pulex* , *Tunga* , and *Xenopsylla* , with those of the species *Ctenocephalides canis* , *Ctenocephalides felis* , *Diamanus montanus* , *Echidnophaga gallinacea* , *Nosopsyllus faciatus* , *Pulex irritans* , *Pulex simulans* , *Tunga penetrans* and *Xenopsylla cheopis* being more preferred, with *C. felis* being even more preferred. Such fleas are also preferred for the isolation of proteins or nucleic acid molecules of the present invention.

[0048] Another embodiment of the present invention is an isolated nucleic acid molecule comprising a flea octopamine receptor nucleic acid molecule, i.e. a nucleic acid molecule that can be isolated from a flea cDNA library. The identifying characteristics of such nucleic acid molecules are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural flea octopamine receptor gene or a homologue thereof, the latter of which is described in more detail below. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another nucleic acid molecule. As such, the minimal size of a flea octopamine receptor nucleic acid molecule of the present invention is from 12 to 18 nucleotides in length.

[0049] In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of

either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. Isolated flea octopamine receptor nucleic acid molecules of the present invention, or homologues thereof, can be isolated from a natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated flea octopamine receptor nucleic acid molecules, and homologues thereof, can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode a flea octopamine receptor protein of the present invention.

[0050] A flea octopamine receptor nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., *ibid*., which is incorporated by reference herein in its entirety. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologues can be selected by hybridization with flea octopamine receptor nucleic acid molecules or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a flea octopamine receptor protein or to effect flea octopamine receptor activity).

[0051] An isolated flea octopamine receptor nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one flea octopamine receptor protein of the present invention respectively, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a flea octopamine receptor protein.

[0052] In one embodiment of the present invention, a preferred flea octopamine receptor nucleic acid molecule includes an isolated nucleic acid molecule that hybridizes under conditions that preferably allow less than or equal to 30% base pair mismatch, preferably under conditions that allow less than or equal to 20% base pair mismatch,preferably under conditions that allow less than or equal to 10% base pair mismatch, preferably under conditions that allow less than or equal to 8% base pair mismatch, preferably under conditions that allow less than or equal to 5% base pair mismatch or preferably under conditions that allow less than or equal to 2% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[0053] One embodiment of the present invention includes a flea octopamine receptor nucleic acid molecule, wherein said nucleic acid molecule hybridizes under conditions comprising, (a) hybridizing in solution comprising 1X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of 37 ° C and (b) washing in a solution comprising 1X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of 74.6 ° C, to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[0054] Additional preferred flea octopamine receptor nucleic acid molecules of the present invention include nucleic acid molecules comprising a nucleic acid sequence at least 35 nucleotides in length that is preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 92%, preferably at least 95%, or preferably at least 98% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11 and encodes a protein that binds octopamine.

[0055] One preferred nucleic acid molecule of the present invention includes at least a portion of nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13, as well as allelic variants of nucleic acid molecules having these nucleic acid sequences and homologues of nucleic acid molecules having these nucleic acid sequences; preferably

such a homologue encodes or is complementary to a nucleic acid molecule that encodes at least one epitope that elicits an immune response against a protein having an amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13. Such nucleic acid molecules can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, a full-length gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent protective compound.

[0056] One embodiment of the present invention is a nucleic acid molecule comprising an isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13; and (b) a nucleic acid molecule having an at least 35 contiguous nucleotide portion identical in sequence to an at least 35 contiguous nucleotide portion of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[0057] In one embodiment, a flea octopamine receptor nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95%, preferably at least 98%, preferably at least 99%, or preferably at least 100% identical to SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12. The present invention also includes a flea octopamine receptor nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, as well as allelic variants of a nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

[0058] In another embodiment, a preferred flea octopamine receptor nucleic acid molecule of the present invention comprises a nucleic acid molecule comprising at least 35 nucleotides, preferably at least 40 nucleotides, preferably at least 45 nucleotides, preferably at least 50 nucleotides, preferably at least 75 nucleotides,

preferably at least 100 nucleotides, preferably at least 125 nucleotides, preferably at least 150 nucleotides, preferably at least 175 nucleotides, preferably at least 200 nucleotides, preferably at least 250 nucleotides, preferably at least 350 nucleotides, preferably at least 400 nucleotides, preferably at least 450 nucleotides, preferably at least 500 nucleotides, preferably at least 550 nucleotides, preferably at least 600 nucleotides, preferably at least 650 nucleotides, preferably at least 700 nucleotides, preferably at least 750 nucleotides, preferably at least 1000 nucleotides, preferably at least 1500 nucleotides, preferably at least 1750 nucleotides, preferably at least 2000 nucleotides or preferably at least 2050 nucleotides in length and encodes a protein that binds octopamine.

[0059] In another embodiment, a preferred flea octopamine receptor nucleic acid molecule encodes a protein comprising at least 180 amino acids, preferably at least 200 amino acids, preferably at least 225 amino acids, preferably at least 250 amino acids, preferably at least 300 amino acids, preferably at least 350 amino acids, preferably at least 400 amino acids, preferably at least 450 amino acids, preferably at least 500 amino acids, preferably at least 550 amino acids, preferably at least 600 amino acids, preferably at least 650 amino acids, or preferably at least 690 amino acids.

[0060] In another embodiment, a preferred flea octopamine receptor nucleic acid molecule of the present invention comprises an apparently full-length flea octopamine receptor coding region, i.e., the preferred nucleic acid molecule encodes an apparently full-length flea octopamine receptor protein, respectively, or a post-translationally modified protein thereof. In one embodiment, a preferred flea octopamine receptor nucleic acid molecule of the present invention encodes a mature protein or extracellular domain.

[0061] In another embodiment, a preferred flea octopamine receptor nucleic acid molecule of the present invention comprises a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13, or a fragment thereof.

[0062] A flea octopamine receptor nucleic acid molecule of the present invention preferably comprises at least 35 nucleotides, preferably at least 40 nucleotides,

preferably at least 45 nucleotides, preferably at least 50 nucleotides, preferably at least 75 nucleotides, preferably at least 100 nucleotides, preferably at least 125 nucleotides, preferably at least 150 nucleotides, preferably at least 175 nucleotides, preferably at least 200 nucleotides, preferably at least 250 nucleotides, preferably at least 350 nucleotides, preferably at least 400 nucleotides, preferably at least 450 nucleotides, preferably at least 500 nucleotides, preferably at least 550 nucleotides, preferably at least 600 nucleotides, preferably at least 650 nucleotides, preferably at least 700 nucleotides, preferably at least 750 nucleotides, preferably at least 1000 nucleotides, preferably at least 1500 nucleotides, preferably at least 1750 nucleotides or preferably at least 2000 nucleotides identical in sequence to a corresponding contiguous sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[0063] The phrase, a nucleic acid molecule comprising at least x contiguous, or consecutive nucleotides identical in sequence to at least x contiguous, or consecutive nucleotides of a nucleic acid molecule selected from the group consisting of SEQ ID NO:y, refers to an x-nucleotide in length nucleic acid molecule that is identical in sequence to an x-nucleotide portion of SEQ ID NO:y, as well as to nucleic acid molecules that are longer in length than x. The additional length may be in the form of nucleotides that extend from either the 5' or the 3' end(s) of the contiguous identical x-nucleotide portion. The 5' and/or 3' extensions can include one or more extensions that have no identity to a molecule of the present invention, as well as extensions that show similarity or identity to cited nucleic acids sequences or portions thereof.

[0064] Knowing the nucleic acid sequences of certain flea octopamine receptor nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other flea octopamine receptor nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention;

traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources to screen or from which to amplify nucleic acid molecules include cDNA and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid*.

[0065] One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of flea octopamine receptor nucleic acid molecules of the present invention.

[0066] One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, insect and mammalian cells, and more preferably in the cell types disclosed herein.

[0067] In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences that control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those that function in bacterial, yeast, or insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7 *lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with fleas, such as *C. felis* transcription control sequences. Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein.

[0068] Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed flea

octopamine receptor protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

[0069] Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. It is to be noted that a cell line refers to any recombinant cell of the present invention that is not a transgenic animal. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include flea octopamine receptor nucleic acid molecules disclosed herein.

[0070] Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the

present invention either can be endogenously (i.e., naturally) capable of producing flea octopamine receptor proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), other insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, insect and mammalian cells. More preferred host cells include *Drosophila melanogaster* S2 cells, *Salmonella* , *Escherichia* , *Bacillus* , *Caulobacter* , *Listeria* , *Saccharomyces* , *Pichia, Spodoptera* , *Mycobacteria* , *Trichoplusia* , BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli* , including *E. coli* K-12 derivatives; *Salmonella typhi* ; *Salmonella typhimurium* , including attenuated strains such as UK-1 π 3987 and SR-11 π 4072; *Caulobacter* ; *Pichia* ; *Spodoptera frugiperda* ; *Trichoplusia ni* ; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK ^{31} cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

[0071] A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell.

[0072] A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred

nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein.

[0073] Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including flea octopamine receptor nucleic acid molecules encoding one or more proteins of the present invention and one or more other nucleic acid molecules encoding other protective compounds, as disclosed herein (e.g., to produce multivalent vaccines).

[0074] Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

[0075] Isolated flea octopamine receptor proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a

recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective, medium refers to any medium in which a cell is cultured to produce a flea octopamine receptor protein of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

[0076] Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

[0077] The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

[0078] The present invention also includes isolated (i.e., removed from their natural

milieu) antibodies that selectively bind to a flea octopamine receptor protein of the present invention or a mimetope thereof (e.g., anti-flea octopamine receptor antibodies). As used herein, the term "selectively binds to" a protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid*., and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*; Cold Spring Harbor Labs Press; Harlow et al., *ibid*., is incorporated by reference herein in its entirety. An anti-flea octopamine receptor antibody of the present invention preferably selectively binds to a flea octopamine receptor protein, respectively, in such a way as to inhibit the function of that protein.

[0079] Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

[0080] One embodiment of the present invention is a therapeutic composition that, when administered to an animal susceptible to flea infestation, is capable of protecting that animal from flea infestation. Therapeutic compositions of the present invention include at least one of the following protective molecules: an isolated flea octopamine receptor protein; a mimetope of an isolated flea octopamine receptor protein; an isolated flea octopamine receptor nucleic acid molecule; and/or a compound derived from said isolated flea octopamine receptor protein that inhibits flea octopamine receptor protein activity, an anti-flea octopamine receptor antibody, and/or a compound that inhibits flea octopamine receptor activity. A therapeutic composition of the present invention can further comprise a component selected from the group of an excipient, a carrier, and/or an adjuvant; these components are described further herein. As used herein, a protective molecule or protective compound refers to a compound that, when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent flea infestation. Preferred fleas to target are heretofore disclosed. One example of a protective molecule is a vaccine or therapy,

such as, but not limited to, a naked nucleic acid vaccine or therapy, a recombinant virus vaccine or therapy, a recombinant cell vaccine or therapy, and a recombinant protein vaccine or therapy. Another example of a protective molecule is a compound that inhibits flea octopamine receptor protein activity, such as an isolated antibody that selectively binds to a flea octopamine receptor protein, a substrate analog of a flea octopamine receptor protein, anti-sense-, triplex formation-, ribozyme-, and/or RNA drug-based compounds, or other inorganic or organic molecules that inhibit flea octopamine receptor protein activity. Inhibiting flea octopamine receptor protein activity can refer to the ability of a compound to reduce the activity of flea octopamine receptor proteins. Inhibiting flea octopamine receptor protein activity can also refer to the ability of a compound to reduce the amount of flea octopamine receptor protein in a flea.

[0081] Another embodiment of the present invention includes a method to reduce flea infestation in an animal susceptible to flea infestation. Such a method includes the step of administering to the animal a therapeutic molecule comprising a protective compound selected from the group consisting of (a) an isolated flea octopamine receptor protein; (b) a mimetope of an isolated flea octopamine receptor protein; (c) an isolated flea octopamine receptor nucleic acid molecule; and (d) a compound derived from an isolated flea octopamine receptor protein that inhibits flea octopamine receptor protein activity.

[0082] As used herein, the term derived, or the term derived from, refers to a peptide, antibody, mimetope, nucleic acid molecule, or other compound that was obtained directly or indirectly from a flea octopamine receptor protein or nucleic acid molecule of the present invention, e.g. a part of a protein or nucleic acid molecule or produced using a protein or nucleic acid molecule of the present invention. Methods to obtain derivatives from a flea octopamine receptor molecule of the present invention are known in the art, and as such include, but are not limited to molecular modeling of flea octopamine receptor proteins to determine active sites, and predicting from these active sites smaller fragments and/or mimetopes that retain and/or mimic these active sites, thereby inhibiting flea octopamine receptor protein activity. Other inhibitors of flea octopamine receptor activity can also be obtained in a variety of ways, including but not limited to screening of peptide or small chemical compound

libraries against flea octopamine receptor proteins of the present invention; and screening of polyclonal or monoclonal antibodies to find antibodies that specifically bind flea octopamine receptor proteins of the present invention.

[0083] A flea octopamine receptor protein inhibitor of the present invention (i.e. an inhibitor of a flea octopamine receptor protein) is identified by its ability to mimic, bind to, modify, or otherwise interact with, a flea octopamine receptor protein, thereby inhibiting the activity of a natural flea octopamine receptor protein. Suitable inhibitors of flea octopamine receptor protein activity are compounds that can inhibit flea octopamine receptor protein activity in at least one of a variety of ways: (a) by binding to or otherwise interacting with or otherwise modifying flea octopamine receptor protein sites; (b) by binding to or otherwise interacting with or otherwise modifying flea octopamine receptor protein active site(s); (c) by binding to the flea octopamine receptor protein and thus reducing the availability of the flea octopamine receptor protein in solution; (d) by mimicking a flea octopamine receptor protein; and (e) by interacting with other regions of the flea octopamine receptor protein to inhibit flea octopamine receptor protein activity, for example, by allosteric interaction.

[0084] Flea octopamine receptor protein inhibitors can be used directly as compounds in compositions of the present invention to treat animals as long as such compounds are not harmful to host animals being treated. Preferred flea octopamine receptor protein inhibitors of the present invention include, but are not limited to, flea octopamine receptor protein substrate analogs, and other molecules that bind to a flea octopamine receptor protein (e.g., to an allosteric site) in such a manner that the activity of the flea octopamine receptor protein is inhibited. A flea octopamine receptor protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the active site of a flea octopamine receptor protein. A preferred flea octopamine receptor protein substrate analog inhibits flea octopamine receptor protein activity. Flea octopamine receptor protein substrate analogs can be of any inorganic or organic composition. Flea octopamine receptor protein substrate analogs can be, but need not be, structurally similar to a flea octopamine receptor protein natural substrate as long as they can interact with the active site of that flea octopamine receptor protein. Flea octopamine receptor protein substrate analogs can be designed using computer-generated structures of flea octopamine receptor

proteins of the present invention or computer structures of flea octopamine receptor protein"s natural substrates. Preferred sites to model include one or more of the active sites of flea octopamine receptor proteins. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic compounds, or other inorganic or organic molecules, and screening such samples for their ability to interfere with interaction between flea octopamine receptor proteins and their substrates, e.g. by affinity chromatography techniques. A preferred flea octopamine receptor protein substrate analog is a flea octopamine receptor protein mimetic compound, i.e., a compound that is structurally and/or functionally similar to a natural substrate of a flea octopamine receptor protein of the present invention, particularly to the region of the substrate that interacts with the flea octopamine receptor protein active site, but that inhibits flea octopamine receptor protein activity upon interacting with the flea octopamine receptor protein active site.

[0085] The present invention also includes a therapeutic composition comprising at least one protective molecule of the present invention in combination with at least one additional compound protective against one or more infectious agents and/or infestation by one or more ectoparasites.

[0086] In one embodiment, a therapeutic composition of the present invention can be used to protect an animal from flea infestation by administering such composition to a flea in order to prevent infestation. Such administration to the flea and/or animal could be oral, or by application to the animal"s body surface (e.g. topical spot-on, or spraying onto the animal), or by application to the environment (e.g., spraying). Examples of such compositions include, but are not limited to, transgenic vectors capable of producing at least one therapeutic composition of the present invention. In another embodiment a flea can ingest therapeutic compositions, or products thereof, present on the surface of or in the blood of a host animal that has been administered a therapeutic composition of the present invention.

[0087] In accordance with the present invention, a host animal (i.e., an animal that is or is capable of being infested with fleas) is treated by administering to the animal a therapeutic composition of the present invention in such a manner that the composition itself (e.g., a flea octopamine receptor protein inhibitor, a octopamine

receptor protein synthesis suppressor (i.e., a compound that decreases the production or half-life of a octopamine receptor protein in fleas), a flea octopamine receptor protein mimetope, or a anti-flea octopamine receptor antibody) or a product generated by the animal in response to administration of the composition (e.g., antibodies produced in response to administration of a flea octopamine receptor protein or nucleic acid molecule, or conversion of an inactive inhibitor "prodrug" to an active flea octopamine receptor protein inhibitor) ultimately enters the flea. A host animal is preferably treated in such a way that the compound or product thereof is present on the body surface of the animal or enters the blood stream of the animal. Fleas are then exposed to the composition or product when they feed from the animal. For example, flea octopamine receptor protein inhibitors administered to an animal are administered in such a way that the inhibitors enter the blood stream of the animal, where they can be taken up by feeding fleas or are administered to the animal topically, where they can be taken up by contact with the treated animal.

[0088] In accordance with the present invention, reducing flea octopamine receptor protein activity in a flea can lead to a number of outcomes that reduce flea burden on treated animals and their surrounding environments. Such outcomes include, but are not limited to, (a) reducing the viability of fleas that feed from the treated animal, (b) reducing the fecundity of female fleas that feed from the treated animal, (c) reducing the reproductive capacity of male fleas that feed from the treated animal, (d) reducing the viability of eggs laid by female fleas that feed from the treated animal, (e) altering the blood feeding behavior of fleas that feed from the treated animal (e.g., fleas take up less volume per feeding or feed less frequently), (f) reducing the viability of flea larvae, for example due to the feeding of larvae from feces of fleas that feed from the treated animal, (g) altering the development of flea larvae (e.g., by decreasing feeding behavior, inhibiting growth, inhibiting (e.g., slowing or blocking) molting, and/or otherwise inhibiting maturation to adults), and/or (h) altering or decreasing the ability of fleas or flea larvae to digest a blood meal.

[0089] In order to protect an animal from flea infestation, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting that animal from flea infestation. Therapeutic compositions of the present invention can be administered to animals prior to

infestation in order to prevent infestation (i.e., as a preventative vaccine) and/or can be administered to animals after infestation (i.e. as a therapy).

[0090] Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

[0091] In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), Flt-3 ligand, granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides;

toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax ™ adjuvant (Vaxcel ™ , Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

[0092] In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

[0093] One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ* . Preferred controlled release formulations are biodegradable (i.e., bioerodible).

[0094] A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least 1 month, more preferably for at least 3 months, even more preferably for at least 6 months, even more preferably for at least 9 months, and even more preferably for at least 12 months.

[0095] The efficacy of a therapeutic composition of the present invention to protect an animal from flea infestation can be tested in a variety of ways including, but not

limited to challenge of the treated animal with the fleas to determine whether the treated animal is resistant to infestation. Challenge studies can include direct administration of fleas to the treated animal. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

[0096] As discussed herein, one therapeutic composition of the present invention includes an inhibitor of flea octopamine receptor protein activity, i.e., a compound capable of substantially interfering with the function of a flea octopamine receptor protein. An inhibitor of flea octopamine receptor protein activity, or function, can be identified using flea octopamine receptor proteins of the present invention. A preferred inhibitor of flea octopamine receptor protein function is a compound capable of substantially interfering with the function of a flea octopamine receptor protein and which does not substantially interfere with the function of host animal octopamine receptor proteins. As used herein, a compound that does not substantially inhibit or interfere with host animal octopamine receptor proteins is one that, when administered to a host animal, the host animal shows no significant adverse effects attributable to the inhibition of octopamine receptor and which, when administered to an animal in an effective manner, is capable of protecting that animal from flea infestation.

[0097] One embodiment of the present invention is a method to identify a compound capable of inhibiting flea octopamine receptor protein activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated flea octopamine receptor protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has flea octopamine receptor protein activity, and (b) determining if the putative inhibitory compound inhibits the activity. Flea octopamine receptor protein activity can be determined in a variety of ways known in the art, including but not limited to determining the ability of flea octopamine receptor protein to bind to or otherwise interact with a substrate. Such conditions under which a flea octopamine receptor protein has flea octopamine receptor protein activity include conditions in which a flea octopamine receptor protein has a correct three-dimensionally folded structure under physiologic conditions, i.e. physiologic pH, physiologic ionic concentrations, and

physiologic temperatures, such as a native protein, a mature protein, a soluble protein, transfected cells or viruses. Accordingly, the correct three-dimensionally folded structure could be used to predict inhibitory compounds.

[0098] Putative inhibitory compounds to screen include antibodies (including fragments and mimetopes thereof), putative substrate analogs, and other, preferably small, organic or inorganic molecules. Methods to determine flea octopamine receptor protein activity are known to those skilled in the art, see for example Han et al. 1996, Neuron 16:1127-1135.

[0099] A preferred method to identify a compound capable of inhibiting flea octopamine receptor protein activity includes contacting an isolated flea octopamine receptor protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has flea octopamine receptor protein activity; and determining if the putative inhibitory compound inhibits the activity.

[0100] A preferred method to identify a compound capable of inhibiting flea octopamine receptor protein activity includes contacting a recombinant cell comprising an isolated flea octopamine receptor protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has flea octopamine receptor protein activity; and determining if the putative inhibitory compound inhibits the activity.

[0101] Another embodiment of the present invention is an assay kit to identify an inhibitor of a flea octopamine receptor protein of the present invention. This kit comprises an isolated flea octopamine receptor protein of the present invention, and a means for determining inhibition of an activity of flea octopamine receptor protein, where the means enables detection of inhibition. Detection of inhibition of flea octopamine receptor protein identifies a putative inhibitor to be an inhibitor of a flea octopamine receptor protein. Means for determining inhibition of a flea octopamine receptor protein include, for example, an assay system that detects binding of a putative inhibitor to a flea octopamine receptor molecule, and an assay system that detects interference by a putative inhibitor of the ability of flea octopamine receptor protein to bind octopamine. Means and methods are described herein and are known

to those skilled in the art.

[0102] The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention. The following examples include a number of recombinant DNA and protein chemistry techniques known to those skilled in the art; see, for example, Sambrook et al., *ibid*.

Example 1

[0103] This example describes the preparation of flea genomic DNA and a flea head and nerve cord cDNA pool by Rapid Amplification of cDNA Ends (RACE pool).

[0104] Due to the rarity of biogenic amine receptors as cDNA, genomic DNA was required for the initial isolation of octopamine receptor sequences. Genomic DNA was isolated from adult fleas as follows. Approximately 100 mg of adult *C. felis* were crushed in a mortar & pestle using DNAzol™ reagent, available from Life Technologies, Rockville, MD, and genomic DNA was recovered according to manufacturer"s instructions. Resultant DNA was resuspended in water.

[0105] A flea head and nerve cord RACE pool was constructed as follows. Approximately 140 female and 60 male adult *C. felis* heads and nerve cords were dissected from unfed fleas then ground to a homogenized powder by mortar and pestle in liquid nitrogen. Total RNA was isolated from the resulting homogenized powder using a modification of the acid–guanidinium–phenol–chloroform method described by Chomczynski et al., 1987, Anal. Biochem. 162, p. 156–159, which is incorporated herein by reference in its entirety. The modification of the method is that solution D described by Chomczynski et al. was changed to a solution of 4 M guanidine isothiocyanate, 25 mM Sodium Citrate pH 7.0, 1.5% Sarcosyl, 0.5 M 2–mercaptoethanol. Spectrophotometer and ethidium bromide stained denaturing gel analyses indicated that the yield of total RNA was approximately 27 μ g. Approximately 6 μ g of total RNA was used as template to construct a RACE pool using a Marathon cDNA Amplification Kit, available from Clontech Laboratories, Inc., Palo Alto, CA, according to the manufacturer's instructions.

Example 2

[0106] This example describes the cloning, sequencing of flea octopamine receptor nucleic acid molecules.

[0107] Degenerate PCR primers designed using information from *Drosophila melanogaster* octopamine receptor OAMB were used to amplify an approximately 150 nucleotide product from genomic DNA of adult, unfed fleas prepared as described above. Forward primer 5' GTNGAYGTNT GGATGTGYAC 3', designated SEQ ID NO:14, was used in combination with reverse primer 5' TGGNGGRAAR CADATNAC 3', designated SEQ ID NO:15, in a PCR reaction using 50 ng of genomic DNA, 2.5 units (U) AmpliTaq polymerase, available from PE Biosystems, Foster City, CA, 0.5U Pfu polymerase, available from Stratagene, La Jolla, CA, 0.2 mM dNTP"s and 0.5 to 1.0 μ M primers, in a total reaction volume of 50 μ L. The following amplification conditions were used: (1) one cycle of 95 ° C for 10 seconds, (2) five cycles of 94 ° C for 10 seconds, 52 ° C for 30 seconds, and 72 ° C for 30 seconds, (3) thirty cycles of 94 ° C for 10 seconds, 49 ° C for 20 seconds, and 72 ° C for 30 seconds. The resulting product, referred to as $nCfOCR_{111}$, was sequenced to reveal a 111 nucleotide product, having a coding strand designated SEQ ID NO:1 and a complementary strand designated SEQ ID NO:2.

[0108] First and second PCR reactions were performed on a flea cDNA library under the following reaction conditions: 2.5 U AmpliTaq polymerase per reaction, 0.2 mM dNTP"s, and 0.5 to 1.0 μ M primers, in a total reaction volume of 50 μ L, were used under the following cycling conditions: (1) one cycle of 95 ° C for one minute, (2) five cycles of 94 ° C 10 seconds, 62 ° C 30 seconds, and 72 ° C for two minutes thirty seconds, (3) ten cycles of 94 ° C for 10 sec, 59 ° C for 30 seconds, and 72 ° C for three minutes, (4) fifteen cycles of 94 ° C for 10 sec, 56 ° C for 30 seconds, and 72 ° C for three minutes. In the first PCR reaction, a forward primer designed using the sequence information obtained from SEQ ID NO:1, having the sequence 5' ATGTGTGGAT GTGTACAGCT TC 3', designated SEQ ID NO:16 was used in combination with a primer designed to anneal to the 3' end of the vector region common to all cDNAs in the library, having the sequence 5' GTAATACGAC TCACTATAGG GC 3', designated SEQ ID NO:17. Three μ L of a flea mixed instar cDNA library, prepared as described in U.S. Patent No. 6,063,610 was used as template. Three μ L of the resulting reaction product were used in a second PCR reaction using SEQ ID NO:17 as

the reverse primer in combination with a forward primer designed using the sequence information obtained from SEQ ID NO:1, having the sequence 5' AAATCTGTGC GCAATATCCT TGG 3', designated SEQ ID NO:18. The resulting PCR product was excised from an agarose gel and T/A cloned using the TOPO T/A ™ cloning kit, available from Invitrogen, Carlsbad, CA. The purified product, denoted $nCfOCR_{2061}$, was sequenced and shown to contain 2061 base pairs, having a coding strand designated SEQ ID NO:3 and a complementary strand designated SEQ ID NO:5. Sequence analysis of SEQ ID NO:3 indicates that $nCfOCR_{2061}$ encodes a protein denoted $PCfOCR_{559}$, having a sequence represented by SEQ ID NO:4, assuming an open reading frame extending from nucleotide 3 through nucleotide 1679 of SEQ ID NO:3. Sequence analysis further demonstrated that SEQ ID NO:4 represents the C-terminus of a flea octopamine receptor.

[0109]

A PCR reaction was performed to isolate the 5' portion of a flea octopamine receptor cDNA from the flea head and nerve cord RACE pool prepared as described in Example 1, as follows. Forward primer AP1, which corresponds to sequence within the adapter flanking the termini of all fragments in the flea head and nerve cord RACE pool, having nucleotide sequence 5' CCATCCTAAT ACGACTCACT ATAGGGC 3', designated SEQ ID NO:19, was used in combination with a reverse primer designed using the sequence information obtained from SEQ ID NO:1, having nucleotide sequence 5' GGAAGCAGAT CACAAAACTA AG 3', designated SEQ ID NO:20. The following PCR conditions were used: 2 U/50 μ L reaction of AmpliTaq polymerase, 0.5 U Pfu polymerase, 0.2mM dNTP"s, 0.5 μ M primers and 3 μ L of a 1/250 dilution of the flea head & nerve cord RACE pool as the template, in a total reaction volume of 50 μ L. Template DNA was added directly to the tubes in the PCR machine after the initial cycling temperature reached 72 ° C . The following amplification conditions were used (1) one cycle of 95 ° C for 1 minute, (2) five cycles of 94 ° C for 10 seconds, 58 ° C for 30 seconds and 72 ° C for 2 minutes, (3) thirty cycles of 94 ° C for 10 seconds, 54 ° C for 30 seconds, and 72 ° C for 2 minutes and 30 seconds. The resulting PCR product was excised from an agarose gel and DNA purified using a QiaQuick ™ Extraction Kit, available from Qiagen, Chatsworth, CA. Two μ L of this product was used as template for nested PCR with forward primer SEQ ID NO:19 and a reverse primer having nucleotide sequence 5' CCAAAGCCCG GCTATGAGTC CC 3', designated SEQ ID NO:21

using the reaction conditions set forth for the primary reaction. The following amplification conditions were used (1) one cycle of 95 ° C for 1 minute, (2) five cycles of 94 ° C for 10 seconds, 58 ° C for 30 seconds and 72 ° C for 1 minute, (3) thirty cycles of 94 ° C for 10 seconds, 54 ° C for 30 seconds, and 72 ° C for 1 minute. The purified product, denoted $nCfOCR_{868}$, was sequenced and shown to contain 868 base pairs, having a coding strand designated SEQ ID NO:6 and a complementary strand designated SEQ ID NO:8. Sequence analysis of SEQ ID NO:6 indicates that $nCfOCR_{868}$ encodes a protein denoted $PCfOCR_{178}$, having a sequence represented by SEQ ID NO:7, assuming an open reading frame extending from nucleotide 333-866 of SEQ ID NO:6. Sequence analysis further demonstrated that SEQ ID NO:7 represents the N-terminus of a flea octopamine receptor.

[0110] Sequence information from SEQ ID NO:3 and SEQ ID NO:6 was used to design primers to amplify one contiguous piece of DNA encoding the entire open reading frame of the nucleic acid molecule encoding a flea octopamine receptor and a PCR reaction was conducted as follows. A forward primer having nucleotide sequence 5' AA *GAATTC* GA TATGAATGCC TCGGAGTACA TTAACACG 3', designated SEQ ID NO:22 and having an *Eco* RI site indicated in bold was used in conjunction with a reverse primer having nucleotide sequence, 5' TT *CTCGAG* CC TCTTGTGACA TCATTATCAC TATCTTG 3', designated SEQ ID NO:23 and having a *Xho* I site indicated in bold. The following reaction conditions were used: 2.5 U PfuTurbo ™ polymerase per reaction and the manufacturers polymerase reaction buffer, available from Stratagene, 3 μ L of a 1/50 dilution of flea head and nerve cord RACE pool as template, 0.2 mM dNTP, and 0.5 μ M primers, in a total reaction volume of 50 μ L. The following cycling conditions were used: (1) one cycle of 94 ° C for 30 seconds, (2) five cycles of 94 ° C for 10 seconds, 53 ° C for 30 seconds, and 72 ° C for 2 minutes, (3) thirty-six cycles of 94 ° C for 10 seconds, 55 ° C for 20 seconds, and 72 ° C for 2 minutes and 30 seconds. The resulting approximately 2 Kb PCR product was excised from an agarose gel as described above and T/A cloned using a TOPO T/A cloning kit. The purified product was sequenced and shown to contain 2082 base pairs, having a coding strand designated SEQ ID NO:9 and a complementary strand designated SEQ ID NO:10.

[0111] A review of SEQ ID NOs. 3, 6, 9 and 10 revealed a sequence discrepancy in SEQ ID NOs. 9 and 10, possibly due to a PCR error, which resulted in an internal stop where a

"GGA" codon had been replaced with a "TGA" codon. PCR mutagenesis was performed to correct the error in SEQ ID NO:9 by a standard method known as PCR overlap extension, as follows. A first PCR reaction was performed using a forward primer having the sequence 5' CAGAGCTATC AACCAA *GGA* T TCAGGACCAC AAAAGG 3', designated SEQ ID NO:24 and having a mutagenized region indicated in bold, was used in combination with a reverse primer corresponding to a region of vector sequence, having the sequence 5' CTTGGTACCG AGCTCGGATC C 3', designated SEQ ID NO:25. A second PCR reaction was performed using a forward primer having the sequence 5' CCTTTTGTGG TCCTGAA *TCC* TTGGTTGATA GCTCTG 3', designated SEQ ID NO:26 and having a mutagenized region indicated in bold, in combination with a reverse primer corresponding to a region of vector sequence, having the sequence 5' AGATGCATGC TCGAGCGGCC G 3', designated SEQ ID NO:27. Each of these PCR reactions was performed using about 100 ng of a T/A clone containing SEQ ID NO:9 described above as template, 2.5U PfuTurbo polymerase, 0.2 mM dNTP, and 0.5 μ M primers, in a total reaction volume of 50 μ L under the following cycling conditions: (1) one cycle of 95 ° C for 30 seconds, (2) five cycles of 94 ° C for 10 seconds, 56 ° C for 30 seconds, 72 ° C for 1 minute, (3) twenty-seven cycles of 94 ° C for 10 seconds, 59 ° C for 20 seconds, 72 ° C for 2 minutes. These PCR reactions produced approximately 1300 base pair and 800 base pair products, respectively.

[0112] One μ l of each of the 1300 base pair and 800 base pair PCR products described above were mixed and used as template in a final PCR reaction to regenerate a full length repaired version of SEQ ID NO:9, as follows. A forward primer with the sequence 5' ATGAATGCCT CGGAGTACAT TAACACGACA ACAATCAG 3', designated SEQ ID NO:28, was used in conjunction with a reverse primer having the sequence 5' TCATCTTGTG ACATCATTAT CACTATCTTG ACGAACG 3', designated SEQ ID NO:29, in a PCR reaction containing 2.5U PfuTurbo polymerase, 0.2 mM dNTP, and 0.5 μ M primers, in a total reaction volume of 50 μ L, under the following cycling conditions: (1) one cycle of 95 ° C for 30 seconds, (2) one cycle of 72 ° C for 5 minutes, (3) five cycles of 94 ° C for 10 seconds, 56 ° C for 30 seconds, and 72 ° C for 2 minutes, (4) twenty cycles of 94 ° C for 10 seconds, 59 ° C for 20 seconds, and 72 ° C for 2 minutes and 30 seconds.

[0113] The resulting PCR product was excised from an agarose gel and run over a

QiaQuick purification column, and found to contain an approximately 2100 base pair nucleic acid molecule. The resulting eluate was polished to facilitate T/A cloning of the fragment as follows: 43 µ L of the total eluate of 50 µ L was mixed with 5 µ L of AmpliTaq™ PCR buffer, 0.1 µ L of 25 mM dNTP mix, and 1 µ L (5U) of AmpliTaq polymerase and incubated at 72 ° C for 8 minutes. Four µ L of this reaction was used in a TOPO T/A cloning reaction performed as described above. The insert from a resulting T/A clone was sequenced which revealed that the error had been corrected.

[0114] The purified product, denoted $nCfOCR_{2136}$, was sequenced and shown to contain 2136 base pairs, having a coding strand designated SEQ ID NO:11 and a complementary strand designated SEQ ID NO:13. Sequence analysis of SEQ ID NO:11 indicates that $nCfOCR_{2136}$ encodes a protein denoted $PCfOCR_{712}$, having a sequence represented by SEQ ID NO:12, assuming a start codon spanning nucleotide 1 to nucleotide 3 and a final codon spanning nucleotide 2134 to nucleotide 2136 of SEQ ID NO:11.

[0115] Comparison of nucleic acid SEQ ID NO:11 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:11 shared the most similarity, i.e. about 49% with a *D. melanogaster* octopamine receptor nucleic acid molecule, OAMB (GenBank accession number AF065443). Comparison of amino acid sequence SEQ ID NO:12 with amino acid sequences reported in GenBank indicates that SEQ ID NO:12 showed the most similarity, i.e. about 55%, with the *Balanus amphitrite* G-protein coupled receptor (GenBank accession number Q93126).

Example 3

[0116] This example describes the expression of a tagged flea octopamine receptor protein.

[0117]

In order to clone the cDNA encoding SEQ ID NO:12 into the DES™ expression system, available from Invitrogen, which also encodes a C-terminal tag, the following PCR was performed. One µ L of a T/A clone containing SEQ ID NO:11 was used as template in a PCR reaction containing 2.5 U PfuTurbo polymerase, 0.2 mM dNTP"s, and 0.5 µ M each of primer SEQ ID NO:22 and primer SEQ ID NO:23 described above under the following cycling conditions: (1) one cycle of 95 ° C for 30 seconds, (2) five

cycles of 94 ° C for 10 seconds, 55 ° C for 30 seconds, and 72 ° C for 2 minutes, (3) twenty cycles of 94 ° C for 10 seconds 59 ° C for 20 seconds, and 72 ° C for 2 minutes. The resultant nucleic acid molecule was excised from an agarose gel, prepared for cloning with a QiaQuick purification column, cut with enzymes *Eco* R1 and *Xho* 1, re-purified over a QiaQuick column and ligated into a pAC-5.1/V5-His B expression vector, available from Invitrogen, that had also been cut with *Eco* R1 and *Xho* 1. This vector encodes a protein with a His tag and a V5 tag at the carboxyl end. Ligation products were transformed into *E. coli* DH5 ∀ and transformant bacteria were screened for inserts by PCR and a clone containing an insert of the appropriate size was used to transfect *Drosophila* S-2 insect cells using the DES expression system, following manufacturer"s protocols. This system utilizes co-transfection with a plasmid carrying the selectable marker blasticidin. CellFectin liposomal regent, available from Life Technologies, was used to deliver 5 µ g of the above-mentioned plasmid DNA and 0.2 µ g pCoBlast plasmid encoding the blasticidin resistance gene, available as part of the DES expression kit, into the cells. At two days post transfection, the cells were split approximately 1:2 and selection was performed for 5 days through the addition of 30 µ g/mL blasticidin, available from Invitrogen, to the media. Following selection, expansion of the cells was performed without selection in shaker flasks. Cells were lysed in SDS-PAGE gel sample loading buffer and a Western blot was performed on cell lysates following separation by denaturing PAGE gel and blotting to a nitrocellulose membrane using techniques known to those skilled in the art. Anti-V5 antibody directed against a portion of the C-terminal fusion protein of the vector, available from Invitrogen, indicated expression of an approximately 90 kilodalton band, which corresponds to the approximate predicted size of a tagged flea octopamine receptor. A Western blot performed under the same conditions on cell lysates from untransfected cells did not contain a homologous 90 kilodalton band.

[0118] While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims:

[0119] What is claimed is:

Claims

[c1] An isolated nucleic acid molecule selected from the group consisting of:
(a) a nucleic acid molecule at least 35 nucleotides in length that hybridizes with a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:13, under conditions comprising: (1) hybridizing in a solution comprising 2X SSC in the absence of nucleic acid helix destabilizing compounds, at a temperature of 37 ° C; and (2) washing in a solution comprising 1X SSC in the absence of helix destabilizing compounds, at a temperature of 74.6 ° C; wherein said at least 35 nucleotide nucleic acid molecule encodes a protein that binds to octopamine; and
(b) a nucleic acid molecule fully complementary to the nucleic acid molecule of (a).

[c2] The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[c3] The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule encodes a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12.

[c4] A recombinant molecule comprising a nucleic acid molecule as set forth in Claim 1 operatively linked to a transcription control sequence.

[c5] A recombinant virus comprising a nucleic acid molecule as set forth in Claim 1.

[c6] A recombinant cell comprising a nucleic acid molecule as set forth in Claim 1.

[c7] A method to produce a protein encoded by an isolated nucleic acid molecule of Claim 1, said method comprising culturing a cell transformed with a nucleic acid molecule encoding said protein.

[c8] An isolated nucleic acid molecule selected from the group consisting of:
(a) a nucleic acid molecule having a nucleic acid sequence selected from the

group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11, and variants thereof at least 95% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11, wherein said nucleic acid molecule encodes a protein that binds to octopamine;
(b) a nucleic acid molecule comprising a fragment of a nucleic acid molecule of (a), wherein said fragment is at least 35 nucleotides in length; and
(c) a nucleic acid molecule fully complementary to a nucleic acid molecule of (a) or (b).

[c9] The nucleic acid molecule of Claim 8, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:13.

[c10] The nucleic acid molecule of Claim 8, wherein said nucleic acid molecule encodes a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12.

[c11] A recombinant molecule comprising a nucleic acid molecule as set forth in Claim 8 operatively linked to a transcription control sequence.

[c12] A recombinant virus comprising a nucleic acid molecule as set forth in Claim 8.

[c13] A recombinant cell comprising a nucleic acid molecule as set forth in Claim 8.

[c14] A method to produce a protein encoded by an isolated nucleic acid molecule of Claim 8, said method comprising culturing a cell transformed with a nucleic acid molecule encoding said protein.

[c15] An isolated protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, and variants therof that are at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12, wherein said protein variant binds to octopamine.

[c16] The protein of Claim 15, wherein said protein is encoded by a nucleic acid

molecule having a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:11.

[c17] The protein of Claim 15, wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12.

[c18] An isolated antibody that selectively binds to a protein as set forth in Claim 15.

[c19] A method to detect an inhibitor of flea octopamine receptor activity, said method comprising (a) contacting an isolated flea octopamine receptor protein of Claim 15, with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has flea octopamine receptor protein activity, and (b) determining if said putative inhibitory compound inhibits flea octopamine receptor protein activity.

[c20] The method of Claim 19, wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:7 and SEQ ID NO:12.

FLEA OCTOPAMINE RECEPTOR NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

Abstract of Disclosure

The present invention relates to flea octopamine receptor nucleic acid molecules; to flea octopamine receptor proteins encoded by such nucleic acid molecules; to antibodies raised against such proteins; and to compounds that inhibit the activity of such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules, antibodies, and inhibitory compounds. The present invention also includes inhibitory compounds, particularly those that specifically inhibit flea octopamine receptor activity, as well as the use of such compounds to treat animals.